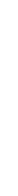
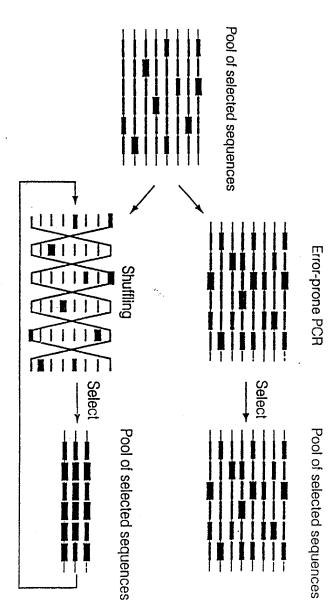




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FIGURE





### **Sexual PCR**

1. Selected mutants

2. Generate random sized fragments of DNA.

3. Becombination and researchly occur in PCR machine.

GAAAAAAA AAAAAAAA AAAAGAAAA AAAAAAAAC

ATAAAAAA



A I'A AA
AAAA GA
AAAA AAA
AAA AAA ନ ≯ AAAA

AAA

AAAAA

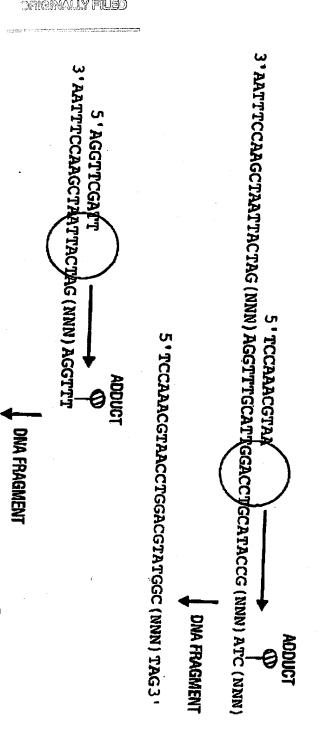


. GTAAGAAAC

FIGURE 2

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- DNA ADDUCTS FOR SEXUAL PCR
- 1. RANDOM PRINTERS ARE USED TO SIMPLIFY TEMPLATES PRETREATED WITH DNA ADDUCTS.
- 2. ADDUCTS CAUSE PREMATURE TERMINATION OF EXTENSION BY BLOCKING THE POLYMERASE. RANDOM SIZE FRAGMENTS ARE CREATED BY RANDOM PRINTING AND PREMATURE TERMINATION,
- 4. DNA FRAGMENTS ARE READY FOR SEXUAL PCR.



5 ' AGGTTCGATTAATGATC (NNN) TCCAA3 '

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## **DNA Adducts**

Aristolochic acid 1
Aristolochic acid 2
2-Amino-3-methylimidazo(4,5-f) quinoline
2-Amino-1-methyl-6-phenylimidazo(4,5-t))pyridine
2-bromoscrolein (2BA)
7-bromoscrolein (2BA)
7-bromoscrolein (2BA)
6-benzo(s)pyrene diolepoxide
benzo(s)pyrene diolepoxide
benzo(s)pyrene
benzo(s)pyrene
benzo(s)pyrene
benzo(s)pyrene
benzo(s)pyrene
benzo(s)pyrene
benzo(s)pyrene
benzo(s)pyrene

## CREATING DNA ADDUCTS USING U.V. LIGHT

1. IRRADIATE POOL OF TEMPLATE DNA WITH U.V. LIGHT,

5'AGATTAAGGAGTCCGTAAGGATT3' 5'AGATTAAGGAGTCCGTAAGGATT3'

5'AGATTAAGGAGTCCGTAAGGATT3'

2. CROSS LINKS IN THE DNA WILL BE INTRODUCED BY THE U.V. THESE CROSS LINKS WILL STOP TAO POLYMERASE EXTENSION.

5 'AGATTAAGGAGTCCGTAAGGATT3'

5 ' Agattaaggagtccgtaaggatt3 '

5 ' agāttaaggagtccgtaaggātt3 '

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3. USE RANDOM PRIMERS ON CROSS LINKED DNA AND EXTEND WITH TAQ POLYMERASE

5 ' Agattaaggagtccgtaaggatt3 ' T 3'AGGCATS

5'AGATTAAGGAGTCCGTAAGGATT3' ↑ 3'CCTAAS

5 ' AGATTAAGGAGTCCGTAAGGATT3 ' T 3'CTCAGS

4. TAO EXTENSIONS ARE BLOCKED BY U.V. ADDUCTS. FOR GENE SHUFFLING THIS CREATES RANDOM SIZE FRAGMENTS READY

3' TCTAATTCCTCAGGCAT5 3'AATTCCTCAG5' 3'AGGCATTCCTAA5'



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# Reassembly of DNA fragments

of mount OCS lane 2- 1kb ladder DNA fragments lane 1- isolated length of ORF is alkaline phosphatuse 44 154 densu HH

reassembly, 1kb amplification, closing is ready for Reassembled product alk phos ORF. Predominant band at of reassembly lane 2-Second round products have formed lanc 1-First round of 1.8kb is the full

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and scaeening.

lanc 3- 1kb ladder

FIGURE 6A

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FIGURE 6B



10

15

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25

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### Figure 7

1					
	R	R			
-	///-	CB-		C→A	C→.
T	he Product - a novel				
				C→A	→C→
: A delet	tion between sequence	1 and seque	nce 3.		
	R		R		
	→A/→	C→/-B		C→A-	C-
r	he Product - a novel	"AB" molec	cule		
	→A	B→	C→	A	C→
	→A		-/→	R /-C→A	R C→
: How m	•	C→B- I "BC" mole	-/→ cule	/-C→A	C→
: How m	→A→ The Product - a novelutiple events can product	C→B- I "BC" mole	-/→ cule	/-C→A	C→
: How m	→A→	C→B- l "BC" mole duce even mo	cule ore complex	c re-assortm	nents:
: How m	The Product - a novelultiple events can product - R  R A/	C→B- l "BC" mole duce even mo	cule  R  -/	c re-assortm	nents:
: How m	The Product - a novelultiple events can product - R  R A/	C→/-B-l "AB" mole	cule  R  -/	c re-assortm	nents:
: How m	The Product - a novelultiple events can product - R  R A/	C→B-l "BC" mole	cule  R  -/	x re-assortm	nents: C→C→C R

Figure 7. The production of novel molecules by reductive re-assortment in which deletions mediated by consecutive sequences result in the production of novel molecules. The inherent instability of repeated sequences drives this process. Multiple changes can occur within a single repeat unit through the reiterative nature of the drive to reduce the repeated index (RI).